FORM PTO-1390 (REV 10-96) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: IKU0102PUSA

U.S. APPLICATION NO (If known, see 37 C.F.R. 1.5): 09/269703

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP97/03499	01 October 1997 (01.10.97)	02 October 1996 (02.10.96)

TITLE OF INVENTION: METHOD OF SEARCHING FOR PHYSIOLOGICALLY ACTIVE SUBSTANCES AND PROCESS FOR PRODUCING THE SAME

APPLICANT(S) FOR DO/EO/US
Kenji Sakamoto

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. X This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ___ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ___ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ___ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. X A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ___ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. X has been transmitted by the International Bureau.
   c. ___ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ___ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ___ Amendments to the claims of the International Application Under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ___ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ___ have been transmitted by the International Bureau.
   c. ___ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ___ have not been made and will not be made.
8. ___ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. X An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ___ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ___ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. X An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. X A FIRST preliminary amendment.
    ___ A SECOND or SUBSEQUENT preliminary amendment.
14. ___ A substitute specification.
15. ___ A change of power of attorney and/or address letter.
16. X Other items or information: Two (2) Verified Statement (Declaration) Claiming Small Entity Status Forms

"Express Mail" mailing label No.: EJ 124 078 696 US

Date of Deposit: March 31, 1999

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" under 37 C.F.R. 1.10 on the date indicated above and is addressed to: P.O. Box PCT, Assistant Commissioner for Patents, Washington, D.C. 20231

Linda J. Robb

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/269703		IKU0102PUSA

17. X The following fees are submitted:

	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO $840.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $670.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $760.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $970.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $96.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ____ 20 ____ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 00.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	5 - 20 =	0	X $18.00	$ 00.00	
Independent claims	2 - 3 =	0	X $78.00	$ 00.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	$ 00.00	
			TOTAL OF ABOVE CALCULATIONS =	$ 840.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$ 420.00	
			SUBTOTAL =	$ 420.00	
Processing fee of **$130.00** for furnishing the English translation later than ____ 20 X 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$ 130.00	
			TOTAL NATIONAL FEE =	$ 550.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property			+	$ 40.00	
			TOTAL FEES ENCLOSED =	$ 590.00	
				Amount to be: refunded	$
				charged	$

a. X A check in the amount of $ 590.00 to cover the above fees is enclosed.

b. ____ Please charge my Deposit Account No. 02-3978 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. X The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-3978 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

KENJI SAKAMOTO

Serial No.: Unknown

Filed: Herewith

For: METHOD OF SEARCHING FOR PHYSIOLOGICALLY ACTIVE SUBSTANCES AND PROCESS FOR PRODUCING THE SAME

Attorney Docket No.: IKU0102PUSA

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Before examination of the above-identified patent application, please amend the application as follows:

IN THE CLAIMS:

Claim 3, line 2, delete "or 2".

CERTIFICATION UNDER 37 C.F.R. § 1.10

I hereby certify that this correspondence is being deposited on the below date with the United States Postal Service in an envelope as "Express Mail Post Office to Addressee" addressed to: Box PCT, Assistant Commissioner for Patents, Washington, D.C. 20231.

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Date of Deposit: MARCH 31, 1999

LINDA J. Robb
(Type name of person mailing paper)

(Signature of person mailing paper)

REMARKS

The claims have been amended to remove multiple dependencies. Please calculate the required filing fee based on the amended claims.

Respectfully submitted,

KENJI SAKOMOTO

By _____
JAMES N. KALLIS
Reg. No. 41,102
Attorney/Agent for Applicant

Date: March 31, 1999

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09/269703 A7

SO Rec'd PCT/PTO 31 MAR 1999 A7

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant or Patentee: KENJI SAKAMOTO
Serial or Patent No.: _____
Filed or Issued: _____ Attorney Docket No. IKU 0102 PUSA
For: METHOD OF SEARCHING FOR PHYSIOLOGICALLY ACTIVE SUBSTANCES AND PROCESS FOR PRODUCING THE SAME

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS

I hereby declare that I qualify as a small entity for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled:

METHOD OF SEARCHING FOR PHYSIOLOGICALLY ACTIVE SUBSTANCES AND PROCESS FOR PRODUCING THE SAME and described in:

___ the specification filed herewith
___ application Serial No. _____ , filed_____ .
___ Patent No. _____ , issued_____ .
✓ the specification filed on October 1, 1997 as PCT International Application No. PCT/JP97/03499.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 C.F.R. § 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d) or a non-profit organization under 37 C.F.R. § 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[✓] no such person, concern, or organization
[ ] persons, concerns or organizations listed below*

** Note:* Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27).

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NON-PROFIT ORGANIZATION

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NON-PROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying the earliest of

the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

HIDEO NAKOSHI
Name of Individual

Hideo Nakoshi
Signature of Individual

March 26, 1999
Date

09/269703

Rec'd PCT/PTO 31 MAR 1999

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant or Patentee: KENJI SAKAMOTO
Serial or Patent No.:
Filed or Issued: Attorney Docket No. IKU 0102 PUSA
For: METHOD OF SEARCHING FOR PHYSIOLOGICALLY ACTIVE SUBSTANCES AND PROCESS FOR PRODUCING THE SAME

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. §§ 1.9(f) and 1.27(b)) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 C.F.R. § 1.9(c) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled:

METHOD OF SEARCHING FOR PHYSIOLOGICALLY ACTIVE SUBSTANCES AND PROCESS FOR PRODUCING THE SAME and described in:

___ the specification filed herewith
___ application Serial No. _____ , filed_____ .
___ Patent No. _____ , issued_____ .
✓ the specification filed on October 1, 1997 as PCT International Application No. PCT/JP97/03499

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 C.F.R. § 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d) or a non-profit organization under 37 C.F.R. § 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[ ] no such person, concern, or organization
[✓] persons, concerns or organizations listed below*

* *Note:* Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27)

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[✓] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NON-PROFIT ORGANIZATION

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NON-PROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

KENJI SAKAMOTO
Name of Inventor

Kenji Sakamoto
Signature of Inventor

1999 Mar 25
Date

SPECIFICATION

TITLE OF THE INVENTION

Method for searching physiologically active substances and producing the same

BACKGROUND OF THE INVENTION

Field of the Invention:

The present invention relates to a method for searching for a variety of new physiologically active substances and producing the same.

Background Art:

Physiologically active substances of unknown types have been searched, heretofore, by analyzing substances present in body fluids or tissues, and identifying and separating new substances therefrom to determine the physiological activities of said substances.

The prior art method as described above comprises the steps of analyzing components in living organisms, searching and isolating new substances, and identifying the physiological activities thereof. It is obvious, however, that there exist an extremely large number of substances within a living organism, and many physiologically active substances are often present at very low concentrations, thus making it difficult to isolate new substances. Furthermore, because living organisms perform a number of physiological activities, it may also be difficult to identify the physiological activities of a newly isolated substance. As can be appreciated from the above discussion, the prior art methods render it difficult to search and isolate new physiologically active substances.

SUMMARY OF THE INVENTION

One aspect of the present invention is to provide a method for searching, with more efficiency and a certain degree of predictability, new physiologically active substances.

The inventor of the present invention found that there may be cases where identical receptors have two or more sizes, wherein a substance or cell having a functional antagonism is a receptor of a substance present in human body, or wherein a cell or substance having a functional antagonism to cells on which a certain substance A causes some effects is a receptor of said substance A present in human body, and that the amino acid sequence of the missing part or the spliced part has a physiological meaning. For example, calcitonin may be bound to a calcitonin receptor present on osteoclasts to suppress the deossification effected by the osteoclasts, while there are osteoblasts that have functional antagonism against the osteoclasts. Regarding calcitonin receptors, there are already reported the amino acid sequence thereof, but there are reported two or more different types having different sizes. The inventor of the present invention has predicted that, in case of calcitonin receptors of such types having two or more sizes, one domain in the receptor of longer size is spliced thereby creating the receptor of shorter size, and that said spliced domain may cause effects on osteoblasts having effects antagonistic to the effects of osteoclasts on which calcitonin causes effects. Upon examination of the spliced domain after chemically synthesizing the domain, it was confirmed that this new peptide promotes osteogenesis by binding to receptors on the osteoblasts. Such confirmation as above showed the correctness of the foregoing prediction made by the inventor of the present invention and the present invention was made.

In another words, the present invention provides a method for searching for physiological active substances, the method comprising

the steps of identifying an amino acid sequence of receptors being present in two or more sizes for identical receptors, wherein a substance or cell having functional antagonism is a receptor of a substance present in human body or a cell or substance having functional antagonism to cells on which a substance causes effects, and determining which domain in the receptor of longer size is missing in the receptor of shorter size. The present invention also provides a method for producing physiologically active peptides, the method comprising the step of producing the missing domain, or the derivative thereof, identified by the method of present invention as described above.

One aspect of the present invention provides for the first time a method for searching, with efficiency and a certain degree of predictability, for new physiologically active substances. According to another aspect of the present invention, new physiologically active substances may be identified by analyzing receptors of substances associated with functional antagonism, thereby eliminating the necessity of isolating, as before in the prior arts, physiologically active substances present in a small quantity in a sample of human body which includes an extremely large number of components. Furthermore, because the physiological activity of the identified physiologically active substance is associated with said functional antagonism, it may be much easier than before to identify the physiological activities thereof. Therefore, according to one method of the present invention, it is made possible to identify new physiologically active substances in much more efficient way than before.

THE BEST MODE FOR CARRYING OUT THE INVENTION

According to one method of the present invention, focus is made on receptors, wherein a substance or cell having functional antagonism is a receptor of a substance present in human body or a cell or substance

having functional antagonism to cells on which the substance causes some effects. The functional antagonism is a fundamental function for the homeostasis in human body and there are a large number of substances or cells having mutual antagonisms within human body. Examples of receptors, wherein a substance or cell having functional antagonism is the receptors of a substance present in human body or a cell or substance having functional antagonism to cells on which a substance A causes effects is the receptor A present in human body, may be found in many receptors, such as Calcitonin receptors (osteoclasts on which calcitonin acts shows functional antagonism to osteoblasts), glucagon receptors (glucogan having functional antagonism to insulin), somatostatin receptors (somatostatin having functional antagonism to growth hormones), and parathyroid hormone (the hormone having functional antagonism to calcitonin, etc.) and the like. Such receptors include but are not limited to 7-transmembrane type receptors such as Carcitonin receptors.

According to one method of the present invention, the amino acid sequences or the sizes of such receptors are analyzed to find receptors having different sizes and yet being identical receptors. This process may be performed by identifying a plurality of times the amino acid sequences or the sizes of the identified receptors, or by making use of literature if reported therein. Some examples of receptors, wherein there are receptors having two or more types having different sizes while being identical receptors may be found in calcitonin receptors, glucagon receptors, somatostatin receptors, and the like.

After identifying that there are two or more types of receptors having different sizes for identical receptors, the amino acid sequences of these two or more types of receptors are compared with each other to identify which domain in the receptor of longer size is missing and thereby rendering it the receptor of shorter size. The missing domain in the receptor of shorter size is the physiologically active substance having some effects on the functional antagonism. In another words, some of the elements in the spliced structure are

the physiologically active substances. It may be concluded that a physiologically active substance is bound to a receptor whereby some portion of the receptor is spliced and this spliced piece of the receptor shows some physiological activity, such as a controlling effect on the functional antagonism. It was confirmed, for example, that, as shown in the examples below, the missing domain in a calcitonin receptor is bound to a receptor present on a osteoblast thereby promoting osteogenesis.

Since the missing domain identified by the above-described method has physiological activities, a physiologically active substance may be obtained by producing such domain. In most cases, the missing domain comprises peptides of relatively short sizes, and therefore, in such cases, a commercially available peptide synthesizer may be used to easily produce by chemically synthesizing the physiologically active substances. Alternatively, such substances may be produced by using a method of gene engineering type according to known methods.

The physiological activity of the physiologically active substance thus obtained is related to the above-mentioned functional antagonism, and therefore, the activity can be easily confirmed by any of suitable methods applicable depending on each functional antagonism.

It is well-known to those skilled in the Art that, in peptides having physiological activities in general, the physiological activity thereof may be maintained even when some of the amino acids thereof are substituted by other amino acids, or when some amino acids are added, or when some of the amino acids are missing. Therefore, the present invention includes a method for producing a substance which has physiological activities inherent to physiologically active substances comprising the said missing domain, wherein some of the amino acids constituting the missing domain are replaced with other amino acids, or some other amino acids are added to amino acids constituting the missing domain, or some of the amino acids are missing

from the amino acids constituting the missing domain. (Foregoing substance is referred to hereinafter in the present application as "derivative" of the missing domain.) Such a derivative preferably has not less than 70%, and more preferably 90%, of homogeneity with the above-mentioned missing domain.

EXAMPLES

The present invention is more specifically described below with reference to examples. It should be noted, however, that the present invention is not limited to the following examples.

[Example 1]

Identification of the missing domain of calcitonin receptor:

The amino acid sequences of calcitonin receptors are described in Journal of Clinical Investigation, Vol. 90, No. 5 (1992). When the described amino acid sequences of calcitonin receptors described in the above reference are compared, the amino acids at 175th through 190th sequence positions of the amino acid sequence of the longer receptor are missing in the amino acid sequence of the shorter receptor. The amino acid sequence of the missing domain is shown in Sequence 1.

[Example 2] Production of peptides:

By using a commercially available peptide synthesizer, a peptide having the amino acid sequence shown in Sequence 1 was synthesized.

[Example 3] Proliferation promotion of osteoblasts:

ROS cells from a rat, which are osteoblasts, (available from ATCC) were cultured in F10 growth medium containing 10% fetal bovine serum (available from Dainippon Pharmaceutical), and incubated in a

chamber of constant temperature of 37° C under humidified air containing 5% of CO2 gas. Using trypsin treatment, the cells were disseminated into a 24-well culture plate at the rate of 1 x 105 cells/well, and when the colonies became confluent, the medium was replaced with F10 medium having 1% of fetal bovine serum and were cultured for 24 hours. Then the peptide of the present invention produced in Example 2 was dissolved into F10 medium having 1% of fetal bovine serum, which was added to the wells at varying quantities, and the culturing was continued for additional 24 hours. After the culturing, the effect of

promotion of cell proliferation by the peptide was measured by using MTT assay to determine the level of proliferation promotion effect as compared to the samples without any treatment. In this case, MTT assay and the calculation of proliferation promotion ratio were performed as follows: according to the protocol of MTF-Cell-Growth Assay kit commercially available from Funakoshi, Co., Ltd., the substance of the present invention was added to the wells at varying quantities and then it was left for one day and night, followed by counting by using colorimetry the number of living cells, making use of the phenomena where cleavage of MTT (3-4,5 Dimethylthiazol-2YL)-2,5 Diphenyl Tetrazolium bromide to dark blue formazan by enzymes present in the mitochondria of a living cell. The following results in colorimetric value were obtained by adding varying quantities of the substance of the present invention, while the value for the control group to which no substance of the present invention is added being at 100%. The results are shown in Table 1, below.

Table 1:

Peptide added (μg/well)	Rate of proliferation promotion (%)
0	100.0
0.001	109.6

0.01	110.5
0.1	636.2
1.0	1317.1

As can be seen from Table 1, the peptide identified according to the method of the present invention was confirmed to cause effects in promoting the proliferation for osteoblasts. Thus the peptide of the present invention is thought to cause a increase in bone density, and thus may be useful in the treatment of osteopathy, such as osteoporosis and the like.

[Example 3] Presence on osteoblasts of the receptors of the peptides of the present invention:

The peptides of the present invention was found to have promotional effect of proliferation of osteoblasts, whereby it is estimated that osteoblasts have receptors of the peptides of the present invention. If the receptors are present, the peptide of the present invention may be thought to be a fundamental substance for life, and therefore, it was investigated whether there are receptors on osteoblasts.

The peptide obtained in Example 2 was labeled with biotin, and the peptide of the present invention labeled to a predetermined quantity was added to ROS cells cultured in a similar method as in Example 3, followed by dissolving, to effectuate a competitive reaction, into F10 medium having 10% of fetal bovine serum the peptide of the present invention not labeled and thereafter by adding while the quantity is varied to observe the competitive reaction. The foregoing experimental operation was performed more specifically as follows: the peptide of the present invention was biotinated according to the protocol of Protein Biotin-Labeling kit from SUMILON Co., and a predetermined quantity of the biotinated peptide was added to a predetermined number of cells disseminated in wells, followed by

adding 0 - 0.512 μg/well of non-labeled peptide was added to each well to effectuate a competitive reaction for 6 hours, and thereafter, the cells were rinsed with PBS and the biotinated peptides bound to receptors of the cell surface was reacted with peroxidase labeled by streptoadipin to observe a color development reaction. When any receptor of the peptides of the present invention is present on the cell surface, a competitive reaction with the peptides of the present invention which are not labeled is effected and the color intensity is decreased. The results are shown in Table 2 below:

Table 2:

Added amount of Peptides of Non-labeled type (μg/well)	Ratio to added amount of peptides of labeled type (%)
0	100
0.032	98.4
0.064	86.5
0.128	79.6
0.256	34.1
0.512	29.5

As shown in Table 2, the ratio to the added amount of peptides of labeled type varies depending on the added amount of the peptides of non-labeled type, and therefore, it is obvious that the osteoblasts have receptors for the peptide of the present invention and it is implied that the substance of the present invention plays a fundamental role.

[acute toxicity test]

An acute toxicity test of the peptide produced in Example 2 was performed by using ddy male mice (weight 40-45 grams). The peptide

of the present invention was dissolved into a saline solution (pH 6.0) to administer the solution through the caudal vein, and thereafter, the mice were subjected to observation for 14 days. The dosage was set at 1, 10, and 100 μg/kg. The results are shown in Table 3 below:

Table 3:

Dosage (μg/kg)	Mortality
1	0/5
10	0/5
100	0/5

[Example 4] Identification of the missing domain in Glucagon receptors:

Using the same methodology as in Example 1, the amino acid sequences of Glucagon receptors described in FEBS Letters 351 (1994), pp. 271-275, were compared to identify the amino acid sequence of the domain being present in the receptors of longer size but missing in the receptors of shorter size. The amino acid sequence of the missing domain thus identified is shown in Sequence 2.

[Example 6] Identification of the missing domain in Somatostatin receptors:

Using the same methodology as in Example 1, the amino acid sequences of Somatostatin receptors described in Molecular Pharmacology, 44: pp. 1008-1015 (1993), were compared to identify the amino acid sequence in the domain being present in the receptors of longer size but missing in the receptors of shorter size. The amino acid sequence of the missing domain thus identified is shown in Sequence 3.

SEQUENCE LISTING

SEQ ID NO: 1
SEQUENCE LENGTH:
SEQUENCE TYPE: amino acid
TOPOLOGY: linear
SEQUENCE DESCRIPTION:

Lys Leu Thr Thr Ile Phe Pro Leu Asn Trp Lys Tyr Arg Lys Ala Leu
1 5 10 15

SEQ ID NO: 2
SEQUENCE LENGTH: 27
SEQUENCE TYPE: amino acid
TOPOLOGY: linear
SEQUENCE DESCRIPTION:

Gly Asn Gly Val Val Ser Ala Trp Glu Ala Glu Gly Ala Lys Ser Gly
1 5 10 15
Ser Gly Leu Thr Arg Ala Tyr Thr His Val Pro
20 25

SEQ ID NO: 3
SEQUENCE LENGTH: 12
SEQUENCE TYPE: amino acid
TOPOLOGY: linear
SEQUENCE DESCRIPTION:

Pro Ser Cys Gln Trp Val Gln Ala Pro Ala Cys Gln
1 5 10

WHAT IS CLAIMED IS:

1. A method for searching for physiologically active substances, the method comprising the steps of:

identifying amino acid sequences of receptors having two or more sizes for identical receptors, wherein a substance or cell having a functional antagonism is a receptor of a substance present in human body, or wherein a cell or substance having a functional antagonism to cells on which a certain substance causes effects is a receptor of the substance present in human body, and

identifying which domain in the receptor having a longer size is missing in the receptor having a shorter size.

2. The method according to claim 1, wherein:

said receptors are receptors of 7-transmembrane type.

3. A method for producing physiologically active peptides comprising the step of:

producing the missing domain, or a derivative thereof, identified by the method according to claim 1 or 2.

4. The method according to claim 3, comprising the step of:

producing said missing domain.

5. The method according to claim 4, wherein:

said missing domain is chemically synthesized.

ABSTRACT OF THE DISCLOSURE

A method for searching, with a predetermined predictability and more ease, for new physiologically active substances is disclosed. In one method for searching for physiologically active substance according to the present invention, amino acid sequences of receptors having two or more sizes for identical receptors are identified, wherein a substance or cell having a functional antagonism is a receptor of a substance present in human body, or wherein a cell or substance having a functional antagonism to cells on which a certain substance causes some effects is a receptor of the substance present in human body, and it is identified which domain in the receptor having a longer size is missing in the receptor having a shorter size.

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

Atty. Docket No. JKU 0102 PUSA
First Named Inventor KENJI SAKAMOTO

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD OF SEARCHING FOR PHYSIOLOGICALLY ACTIVE SUBSTANCES AND PROCESS FOR PRODUCING THE SAME,

the specification of which:

[ ] is attached hereto; or

[✓] was filed on (MM/DD/YYYY) 10/01/1997 as U.S. Application Number or PCT International Application Number PCT/JP97/03499, and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	*Country*	*Foreign Priority Date (MM/DD/YYYY)*	*Priority Not Claimed*	*Certified Copy Attached? (Yes/No)*
08/281,421	JP	10/02/1996		No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

Application Number(s)	*Filing Date (MM/DD/YYYY)*

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

Application Number(s)	*Filing Date (MM/DD/YYYY)*	*Status: Patented, Pending, Abandoned*

Declaration for Patent Application (cont'd.)

Atty. Docket No. IKU 0102 PUSA

I hereby appoint the following registered practitioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Emil L. Brooks, Reg. No. 26,260; James A. Kushman, Reg. No. 25,636; David R. Syrowik, Reg. No. 22,956; Mark A. Cantor, Reg. No. 30,614; Ralph M. Burton, Reg. No. 17,768; Robert C. Tuttle, Reg. No. 27,967; Earl J. LaFontaine, Reg. No. 30,766; Ronald M. Nabozny, Reg. No. 28,648; Thomas A. Lewry Reg. No. 30,770; John E. Nemazi, Reg. No. 30,876; Kevin J. Heinl, Reg. No. 29,805; William G. Abbatt, Reg. No. 21,936; Donald J. Harrington, Reg. No. 17,427; Paul M. Schwartz, Reg. No. 33,278; Timothy G. Newman, Reg. No. 34,228; Frederick M. Ritchie, Reg. No. 18,669; Robert C. Brandenburg, Reg. No. 29,048; A. Frank Duke, Reg. No. 20,937; John M. Halan, Reg. No. 35,534; Jeffrey M. Szuma, Reg. No. 35,700; James R. Ignatowski, Reg. No. 26,741; Frenk A. Angileri, Reg. No. 36,733; William G. Conger, Reg. No. 34,209; Rhonda L. McCoy-Pfau, Reg. No. 37,887; Sangeeta G. Shah, Reg. No. 38,614; Christopher W. Quinn, Reg. No. 38,274; Robert C. Jones, Reg. No. 35,209; David S. Bir, Reg. No. 38,383; Konstantine J. Diamond, Reg. No. 39,653; James N. Kallis, Reg. No. 41,102; Hugo A. Delevie, Reg. No. 32,688; Ralph P. Smith Reg. No. 35,474; Michael S. Brodbine, Reg. No. 38,392; Jeremy J. Curcuri, Reg. No. 42,656; Mark D. Chvey, Reg. No. 42,415; and John J. Ignatowski, Reg. No. 36,555; Pete N. Kioulis, Reg. No. 41,117; Gigette M. Bejin, Reg. No. P-44,027; Stephanie M. Mansfield, Reg. No. P-42,773; Mark E. Stuenkel, Reg. No. P-44,364.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of Sole or First Inventor KENJI SAKAMOTO

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Inventor's signature Kenji Sakamoto Date 1999. Mar. 25

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